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16. ABSTRACT

Sheepshead minnows (Cyprinodon variegatus) were exposed to the synthetic pyrethroid insecticides AC 222,705, fenvalerate and permethrin for 28 days in early life stage toxicity tests.

AC 222,705 was 370 times more toxic than permethrin and 30 times more toxic than fenvalerate with survival and size of newly hatched fish being the most sensitive measure of effect. AC 222,705 reduced survival of hatched fish at 0.61 µg/ℓ and average weight at 0.06 µg/ℓ; no effects were detected at 0.03 µg/ℓ. Concentrations of AC 222,705 that diminished weights were less than our limits of chemical detection, 0.15 µg/ℓ. Fenvalerate reduced survival of hatched fish at 3.9 µg/ℓ and both weight and length at 2.2 µg/ℓ; no effects were detected at 0.56 µg/ℓ. Permethrin reduced survival of hatched fish at 22 µg/ℓ and no effects were detected at 10 µg/ℓ. The quotient of the 96-hr LC50 divided by the no-effect concentration was 0.8 for permethrin, 9 for fenvalerate and 37 for AC 222,705.

The mean bioconcentration factors, concentrations measured in whole fish divided by concentrations measured in exposure water, were 480 for permethrin and 570 for fenvalerate; AC 222,705 was not detected in fish that survived the exposure.

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EFFECTS OF THE SYNTHETIC PYRETHROIDS AC 222,705, PERMETHRIN AND FENVALERATE ON SHEEPSHEAD MINNOWS IN EARLY LIFE STAGE TOXICITY TESTS

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Abstract — Sheepshead minnows (*Cyprinodon variegatus*) were exposed to the synthethic pyrethroid insecticides AC 222,705, permethrin or fenvalerate for 28 d in early life stage toxicity tests. AC 222,705 was 370 times more toxic than permethrin and 30 times more toxic than fenvalerate, with survival and size of fish being the most sensitive measures of effect. AC 222,705 significantly reduced survival of hatched fish at 0.61 μg/L and their average weight at 0.06 μg/L; no effects were detected at 0.03 μg/L. Two of the AC 222,705 concentrations that diminished weights, 0.06 and 0.12 μg/L, were below our limit of chemical detection (0.15 μg/L). Permethrin reduced survival of hatched fish at 22 μg/L; no effects were detected at 10 μg/L. Fenvalerate significantly reduced survival of hatched fish at 3.9 μg/L and both weight and length at 2.2 μg/L; no effects were detected at 0.56 μg/L. The 96-h LC_{50} divided by the no-effect concentration was 0.8 for permethrin, 9 for fenvalerate and 37 for AC 222,705. The mean bioconcentration factors determined from concentrations measured in whole fish divided by average concentrations measured in exposure water were 480 for permethrin and 570 for fenvalerate; AC 222,705 was not detected in fish that survived the exposure.

Keywords — AC 222,705 Permethrin Fenvalerate *Cyprinodon variegatus*

INTRODUCTION

Synthetic pyrethroids are manufactured, registered and used as alternatives to organochlorine, organophosphate, carbamate and natural pyrethrum insecticides because they are efficacious, highly toxic to pests and have low-to-intermediate persistence. They are effective in controlling insect pests of cotton and other agricultural crops [1]. They are also effective in controlling biting midges [2], mosquitoes [3–6] and tsetse flies [7] in experimental applications, but application rates sufficient to control mosquitoes and tsetse flies also adversely affected nontarget freshwater organisms.

Synthetic pyrethroids are acutely toxic to aquatic organisms and chronically toxic to freshwater fish, but we are unaware of data on their chronic toxicity to estuarine fish. The 96-h LC_{50} of AC 222,705 to fathead minnows (*Pimephales promelas*) was 0.22 μg/L [8] and ranged from 0.008 to 1.1 μg/L for three species of estuarine fish and invertebrates [9]. The range of LC_{50} values for permethrin exposures lasting 24 to 96 h was 0.39 to 97 μg/L for eight species of freshwater fish and invertebrates [4,10–13] and was 0.02 to 7.8 μg/L for six marine species

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vertebrates [9]. The range of LC_{50} values for permethrin exposures lasting 24 to 96 h was 0.39 to 97 μg/L for eight species of freshwater fish and invertebrates [4,10–13] and was 0.02 to 7.8 μg/L for six marine species [9,14]. The acute toxicity of fenvalerate to five freshwater fishes ranged from 2.1 to 200 μg/L [4,11] and from 0.008 to 5.4 μg/L for five species of estuarine fish and invertebrates [9]. In early life stage toxicity tests with *P. promelas*, no-effect concentrations were 0.03 μg/L for AC 222,705, 0.66 μg/L for permethrin and 0.33 μg/L for fenvalerate [8,15]. The principal objective of our study was to determine the chronic toxicity of these three synthetic pyrethroids to, and their bioconcentration in, the estuarine sheepshead minnow (*Cyprinodon variegatus*) in early life stage toxicity texts.

METHODS

Twenty-eight-day, early life stage toxicity tests using sheepshead minnows were conducted with the synthetic pyrethroid insecticides AC 222,705, permethrin and fenvalerate to determine effects on embryonic development, hatching success and survival and growth of hatched fish. Eggs were obtained from hormone-injected females (11 in the AC 222,705 test and 15 for permethrin) and fertilized by using five or more males, as decribed by Hansen [16]. Sheepshead minnow embryos for the fenvalerate test were obtained using the natural spawning techniques described by Hansen [16]. Chemical analyses of embryos for each test revealed no detectable chlorinated insecticides or polychlorinated biphenyls (<0.1 μg/g) or the pyrethroid to be tested.

All three tests were conducted in an intermittent-flow system using a 1-liter proportional diluter similar to that described by Schimmel et al. [17]. The diluter delivered 0.5 liters of test solution during each cycle, 130 to 260 cycles/d, to each of two replicate aquaria per treatment. Aquaria contained 2.5 liters of solution when full and 1.0 liter of solution following cycling of the self-starting siphon, which ensured water exchange in the two incubation cups in each aquarium. Each pyrethroid was dissolved in triethylene glycol (TEG) and delivered at 0.02 ml/cycle to achieve six toxicant concentrations and a solvent control. Solvent concentrations were constant at 9 mg/L in all treatments. A seawater control was not used because use of both types of controls in more than 10 early life stage tests with this species indicated no consistent TEG-related effects at this concentration.

Each test began with 20 1.5- to 24-h-old embryos that were randomly assigned to each of the four incubation cups per treatment (a total of 80 embryos per treatment). Cups consisted of 9-cm i.d. petri dish bottoms to which a 10-cm high, 450-μm nylon mesh cylinder was attached. Water temperature was maintained at 30 ± 1.5°C, salinity ranged from 22 to 32 ‰ and photoperiod was 12 h. Embryos and hatched fish were examined for survival and fish were fed *Artemia salina* nauplii twice daily, except on weekends when they were fed once daily. Dissolved oxygen was measured weekly in each treatment, and throughout all tests ranged from 3.8 to 6.6 mg/L (58 to 100% of saturation). On the last day of the exposure, day 28, fish in each replicate test were either photographed for subsequent standard-length measurement or towel-dried and weighed to a tenth of a milligram, or both, and frozen for chemical analyses.

Analyses of variance and Duncan's multiple range test were used to determine treatment differences in embryo hatching, juvenile survival, overall survival and effects on weight or length at $\alpha = 0.05$.

Water samples from each treatment were analyzed at least weekly and surviving fish were analyzed at the end of the test using methods described by Schimmel et al. [9]. The source, purity, gas chromatography and mass spectrometry data for each pyrethroid were the same as those listed in Table 2 in that report [9]. The AC 222,705

standard was the same material as used in the test and was not adjusted for purity (reported to be 77% by the manufacturer). Therefore, measured concentrations of this pyrethroid are in micrograms of the technical material per liter. Recoveries from spiked samples analyzed before each analysis ranged from 95% to 110%. Concentrations of each pyrethroid are mean measured concentrations, without corrections for percentage recovery.

RESULTS

AC 222,705

The pyrethroid AC 222,705 significantly reduced the average weight of sheepshead minnows in nominal concentrations ≥ 0.06 μg/L (Table 1). Survival was significantly decreased at the two highest concentrations. Because embryos are generally more tolerant of toxicants than are newly hatched fish, and because the AC 222,705 effects on embryos were not concentration-dependent, we believe that only the deaths of newly hatched fish in measured concentrations ≥ 0.61 μg/L were related to exposure to this pyrethroid. Hatching began on the fourth day (24% hatch) and fish in 0.61 and 0.91 μg/L concentrations began to die on the fifth day. All fish in the 0.91 μg/L concentration were dead by day 7; 10 fish remained alive in the 0.61 μg/L concentration. Six of these 10 fish survived until termination of the test, but were usually inactive. Affected fish were bent and swam in circles. Movement of the caudal area was exaggerated, with the tail appearing to contact the head. Decreases in average weight of surviving fish generally appeared concentration-dependent; fish in the 0.06 μg/L concentration weighed 83% of control weight, fish in 0.12 μg/L, 85% of control, and fish in 0.27 μg/L, 75% of control.

AC 222,705 was not found (detection limit ≥ 0.5 mg/kg) in fish surviving the 28-d exposure. Therefore, the bioconcentration factor (BCF) — concentration in whole fish after the 28-d exposure divided by the concentration in water — at an exposure concentration of 0.27 μg/L was < 1,800.

Permethrin

Permethrin concentrations ≥ 22 μg/L reduced survival of newly hatched sheepshead minnows, but survival in concentrations ≤ 10 μg/L was unaffected (Table 2). Time-to-hatching, survival of embryos to hatching and standard lengths of fish surviving exposure to permethrin were not different from those of the control. Fifty-three percent of

Table 1. Survival and average weight of, and concentration of AC 222,705 in, sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to this pyrethroid insecticide for 28 d at 30 ± 1.5°C and 30 (28.0 – 32.0) ‰ salinity in an early life stage toxicity test

Exposure concentration (μg/L)							
	Average measured (SD)	Survival (%)			Average weight (mg)	Concentration in fish (mg/kg)
Nominal		Embryos	Fry	Combined		
Control	ND[a]	84	100	84	32.1	ND[a]
0.03	ND[a]	66	94	62[d]	30.1	ND[a]
0.06	ND[b]	65	96	62[d]	26.7[d]	ND[a]
0.12	ND[c]	88	99	86	27.4[d]	ND[a]
0.25	0.27 (0.07)	71	98	70	24.2[d]	ND[a]
0.5	0.61 (0.11)	62[d]	13[d]	8[d]	29.1[e]	—
1.0	0.91 (0.05)	68	0[d]	0[d]	—	—

[a]ND, not detectable (< 0.15 μg/L in water, < 0.5 mg/kg in tissue).
[b]AC 222,705 was detectable in two of the five water samples; concentrations averaged 0.20 μg/L.
[c]AC 222,705 was detected at 0.23 μg/L in one of the five water samples.
[d]Significantly different from control ($\alpha = 0.05$).
[e]Excluded from statistical analysis because of high mortality and feeding bias.

Table 2. Survival and standard length of, and concentrations of permethrin in, sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to this pyrethroid insecticide for 28 d at 30 ± 1°C and 25 (22.0 – 28.5) ‰ salinity in an early life-stage toxicity test

Exposure concentration (μg/L)			Survival (%)			Average standard length (mm)	Concentration in fish (mg/kg)	Bioconcentration factor[d]
Nominal	Average measured (SD)		Embryos	Fry	Combined			
Control	ND[a]		95	97	92	9.8	ND[a]	—
1.25	1.6	(0.13)	99	96	95	10.0	0.46	290
2.5	2.4	(0.36)	99	99	98	10.2	1.5	620
5.0	5.6	(0.93)	99	80	79	10.0	2.4	430
10.	10.0	(2.6)	98	99	96	9.8	5.7	570
20.	22.0	(2.9)	96	1[b]	1[b]	15.5[c]	—	—
40.	42.0	(2.1)	99	0[b]	0[b]	—	—	—

[a]ND, not detectable (< 0.25 μg/L in water, < 0.25 mg/kg in tissue).
[b]Significantly different from control ($\alpha = 0.05$).
[c]Excluded from statistical analysis because of high mortality and feeding bias.
[d]Bioconcentration factors are concentration in whole fish/average measured concentration in water.

the fish had hatched by the third day of exposure, but none appeared affected. On the fourth day, 99% had hatched and fish in 22 and 42 μg/L concentrations were visibly affected. Affected fish swam abnormally, their heads moving laterally far in excess of normal. Severely affected fish were lethargic, and some were flexed as much as 90° at midbody. In the 42 μg/L concentration, 64% of the fish were dead on day 5; all were dead on day 6. Fish in the 22 μg/L concentration began to die on day 5 (13% mortality) and all but two (98% mortality) were dead by day 9.

Permethrin was accumulated by sheepshead minnows in proportion to the concentration in exposure water. Bioconcentration factors averaged 480, ranging from 290 to 620 (Table 2).

Fenvalerate

Fenvalerate concentrations ≥ 2.0 μg/L reduced standard lengths of sheepshead minnows, and survival was lessened in concentrations ≥ 3.9 μg/L (Table 3). Survival and lengths were not affected in concentrations ≤ 0.56 μg/L. Time-to-hatching and survival of embryos to hatching were not affected in any concentration tested. Hatching began on the fourth day (13% hatch). Fry began to die by day 5 and mortality had peaked by day 10. Affected fish were lethargic, rested in a curled position and ate

Table 3. Survival, standard length, and weights of, and concentrations of fenvalerate in, sheepshead minnows (*Cyprinodon variegatus*) continuously exposed to this pyrethroid insecticide for 28 d at 30 ± 1.5°C and 26 (23.0 – 30.0) ‰ salinity in an early life stage toxicity test

Exposure concentration (μg/L)			Survival (%)			Average standard length (mm)	Average weight (mg)	Concentration in fish (mg/kg)	Bioconcentration factor[d]
Nominal	Average measured (SD)		Embryos	Fry	Combined				
Control	ND[a]		82	91	75	12.1	27.1	ND[a]	—
0.31	0.28	(0.07)	80	98	79	11.8	24.7	0.13	460
0.62	0.56	(0.10)	89	96	85	11.8	23.9	0.20	360
2.5	2.0	(0.38)	78	94	72	11.3[b]	21.0	1.4	700
1.2	2.2	(0.31)	85	99	84	11.0[b]	17.2[b]	1.1	500
5.0	3.9	(0.88)	58	47[b]	26[b]	12.9[c]	34.4[c]	3.2	820
10.0	7.6	(1.1)	75	10[b]	9[b]	13.9[c]	—	—	—

[a]ND, not detectable (< 0.15 μg/L in water, < 0.10 mg/kg in tissue).
[b]Significantly different from control ($\alpha = 0.05$).
[c]Excluded from statistical analysis because of high mortality and feeding bias.
[d]Bioconcentration factors are concentration in whole fish/average measured concentration in water.

infrequently. Average standard lengths of control fish and fish in 0.28 and 0.56 μg/L concentrations ranged in length from 11.8 to 12.1 mm. Fish in 2.0 and 2.2 μg/L concentrations were significantly shorter, averaging 11.3 and 11.0 mm, respectively. Fish in the 2.2 μg/L concentration also weighed significantly less than control fish. Fish in 3.9 and 7.6 μg/L concentrations were larger than controls, a frequent occurrence in early life stage tests with *C. variegatus* when significant mortality increases the food and space available per fish.

Fenvalerate was accumulated by sheepshead minnows. Bioconcentration factors averaged 570, ranging from 360 to 820 (Table 3).

DISCUSSION

The toxicity of the three pyrethroid insecticides to sheepshead minnows in early life stage toxicity tests differed. The lower chronic value (highest concentration causing no significant effect) and upper chronic value (lowest concentration causing significant adverse effects) ranged from 0.03 to 0.06 μg/L for AC 222,705, from 10 to 22 μg/L for permethrin and from 0.56 to 2.0 μg/L for fenvalerate. Decreased survival was the most sensitive measure of effect in fish exposed to permethrin. In contrast, reduced average length or weight occurred in concentrations of permethrin about one-half that causing mortality with fenvalerate and about one-eighth that causing mortality with AC 222,705. The lower chronic values for fathead minnows in early life stage toxicity tests were 0.03 μg/L for AC 222,705, 0.66 μg/L for permethrin and 0.33 μg/L for fenvalerate [8,15]. Values for AC 222,705 and fenvalerate were almost identical to those for sheepshead minnows in this study. Sheepshead minnows were affected in AC 222,705 concentrations below our limit of detection, but contained no quantifiable tissue concentrations. This suggests that environmental monitoring programs having our detection limit might be incapable of measuring AC 222,705 in water or fish tissue, even though effects might have occurred.

The sheepshead minnow was the most tolerant marine species tested in acute exposures with these pyrethroids [9]; this fish could tolerate exposures at early life stages to concentrations acutely lethal to more sensitive fish and invertebrates (Table 4). In acute exposures, sheepshead minnows were >100 times more tolerant to all three insecticides than were mysids. The difference between the 96-h LC_{50} for the most acutely sensitive marine species and the lowest concentration in our tests in which survival of sheepshead minnows was reduced was 76 for AC 222,705, 1,100 for permethrin and 49 for fenvalerate. Therefore, if environmental concentrations were sufficient to kill only the most sensitive invertebrate species, it would seem unlikely that survival of sheepshead minnows and similarly sensitive fishes would be directly impacted. Instead, indirect effects on the fish by disruption of the food chain, as suggested by Smies et al. [7], might result. AC 222,705 directly affected growth of juvenile fish in our tests in concentrations 8 times the 96-h LC_{50} to mysids. The results of combined direct effects of a chemical on fish growth and possible indirect effects by food chain disruption in the environment are unknown.

The relationship between acute and chronic toxicities to sheepshead minnows of the three pyrethroids differed. The 96-h LC_{50} values and 95% confidence limits from flow-through tests conducted at our laboratory with measured concentrations were 1.1 (range, 0.38 to 1.3) μg/L for AC 222,705, 7.8 (range, 6.2 to 10) μg/L for permethrin and 5.0 (range, 4.8 to 5.3) μg/L for fenvalerate [9] (Table 4). The ratio of the 96-h LC_{50} for permethrin to the lower chronic value (acute chronic ratio) in our test was 0.8. This acute chronic ratio, and the fact that almost all deaths occurred in the first few days after hatching, supports a lack of chronic toxicity for this pyrethroid to sheepshead minnows. The acute chronic ratio was 37 for AC 222,705 and 9 for fenvalerate.

Table 4. Toxicity of AC 222,705, permethrin and fenvalerate to selected estaurine organisms

Measure of effect	Species	Concentration μg/L[a] AC 222,705	Permethrin	Fenvalerate	Source
96 h LC_{50}	*Mysidopsis bahia* (bay mysid)	0.008[b]	0.02[b]	0.008[b]	[9]
	Penaeus duoraum (pink shrimp)	0.22	0.22	0.84	[9]
	Menidia menidia (Atlantic silverside)	—	2.2	0.31	[9]
	Mugil cephalus (striped mullet)	—	5.5	0.58	[9]
	Cyprinodon variegatus (sheepshead minnow)	1.1	7.8	5.0	[9]
Early life stage—no effect concentration	*Cyprinodon variegatus* (sheepshead minnow)	0.032[b]	10	0.56	Present study
	Pimephales promelas (fathead minnow)	0.03	0.66	0.33	[15]

[a]Based on measured concentrations except where otherwise noted.
[b]Based on nominal concentrations because effect concentrations were less than chemical detection limits.

Accumulation of the three pyrethroids by sheepshead minnows was generally less than that observed in other species. Average BCF values in sheepshead minnows were 480 for permethrin, 570 for fenvalerate and < 1,800 for AC 222,705. Schimmel et al. [9] observed steady-state BCF values in eastern oysters (*Crassostrea virginica*) of 2,300 for AC 222,705, 1,900 for permethrin and 4,700 for fenvalerate. The BCF values in the freshwater fathead minnow (*P. promelas*) at the end of an early life stage toxicity test also were greater than those for sheepshead minnows, and averaged 4,000 for AC 222,507, 2,800 for permethrin and 3,200 for fenvalerate [8,15].

Permethrin and fenvalerate have been shown to affect nontarget freshwater organisms at experimental application rates or concentrations necessary to control mosquito larvae or pupae. Applications of permethrin at 0.01 to 0.025 pounds per acre produced 90% to 100% control of five species of flood or stagnant-water mosquito larvae, but eliminated nontarget mayfly (mostly baetids) naiads [3,6]. In addition, applications of fenvalerate at 0.025 to 0.10 pounds per acre produced similar mosquito control, eliminated mayfly naiads and suppressed numbers of dragonfly naiads and diving beetle larvae and adults. Mulla et al. [5] summarized the literature on the impact of pyrethroids on nontarget organisms and reported effects of fenvalerate or permethrin on the species above as well as on copepods, ostracods, caddis flies, gammarid amphipods and backswimmers at application rates required to control mosquitos or simulids. Rates of these experimental applications were similar to rates allowed in registered uses for agricultural applications. Laboratory studies with fenvalerate and permethrin suggest that impact on fish might also be possible since acute LC_{50} and

LC_{90} values to mosquito larvae were within a factor of 4 of the LC_{50} and LC_{90} for some freshwater fishes [4].

Can use of these pyrethroids result in concentrations in the marine environment that could affect nontarget marine organisms? No literature to confirm or refute this possibility was found. Expected uses and application rates for these chemicals, their effects on nontarget freshwater organisms following experimental applications, persistence of the chemicals and their acute and chronic toxicities to freshwater and marine species provide some of the information needed to estimate their potential for environmental impact. Experimental-use permits or conditional registrations have been granted for multiple applications at 0.05 pounds active ingredient per acre for control of cotton pests and, in some instances, pests of other crops [1]. At similar application rates, effects on nontarget freshwater species were observed after single experimental applications to small sites for mosquito control [3,5,6]. In laboratory studies, the pyrethroids we tested that were most acutely toxic to sheepshead minnows also had proportionally greater chronic toxicity and a longer persistence, suggesting a greater potential for environmental hazard. The half-life in estuarine sediment-water systems was < 2.5 d for permethrin, but was 16 d for AC 222,705 and 32 d for fenvalerate [9]. For AC 222,705 and fenvalerate, these durations are sufficient for transport to estuaries where acute or chronic exposure might occur. These three pyrethroids affected survival of marine invertebrate species in acute toxicity tests at concentrations one to two orders of magnitude less than those that affected freshwater species. However, effects seen in our tests on early life stages were generally similar to those seen in similar tests with the fathead minnow. The concentrations that affected marine species were, in some instances, not detectable by our analytical techniques, but were nominally in the low part per trillion (ng/L) range. At this stage of the evaluation of these insecticides, the data suggest a need to continue the hazard evaluation process to emphasize the acquisition of data needed for estimating environmental concentrations and field data to confirm or refute laboratory estimates of environmental concentrations and effects.

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